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Molecular Detection of Extended-Spectrum Beta-lactamase Producing *Salmonella typhi* Isolates in Patients Attending a Tertiary Care Hospital in North-Central Nigeria

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ABSTRACT

Indiscriminate and irrational use of antibiotics has led to the emergence of antibiotic resistance of *Salmonella typhi* (*S. typhi*) and the rapid spread of extended-spectrum beta-lactamase (ESBL) producing strains conferring resistance. This is in fact worrisome, is threatening antibiotic therapy and placing a substantial clinical and financial burden on the healthcare system, patients and their families. Aim of research was to detect molecularly extended-spectrum beta-lactamase producing *S. typhi* isolates in patients attending Bingham University Teaching Hospital (BhUTH), Jos, Plateau State, North-Central Nigeria. A total of 353 stool samples were collected from patients attending BhUTH, and identified using standard microbiological techniques. Antibiotics susceptibility testing was carried out using disc diffusion method, and double disc synergy test (DDST) for phenotypic ESBL production. Polymerase Chain Reaction (PCR) and agarose gel electrophoresis were done to detect the presence of ESBL genes. *S. typhi* prevalence was 37(10.4%). Antibiotics susceptibility profile showed highest resistance to Augmentin and Ampicillin (100%) and less resistance to Nitrofurantoin (0%) and Cefuroxime (24.3%). The *S. typhi* isolates had CRX-AUG-AMP-AMX-STR-CH and CRX-AUG-AMP-AMX-STR as the most frequent resistant phenotypes (10.8%) with multidrug-resistant (MDR) isolates (73%). ESBL producing isolates were 5(13.5%). PCR and agarose gel electrophoresis confirmed the presence of *bla*CTX-M 2(40.0%), *bla*SHV 3(60.0%) and *bla*TEM 5(100.0%) on 857, 615, and 972 base pairs respectively. *S. typhi* isolates showed higher susceptibility to Nitrofurantoin and Cefuroxime. Prompt monitoring of antibiotics usage, resistance to antibiotics and public health education are therefore necessary in order to reduce bacterial disease burden.

Keywords: Molecular detection, *Salmonella typhi*, blaCTX-M, blaSHV, and Antibiotic resistance.

INTRODUCTION:

Prokaryotes (bacteria, yeast, etc) store their DNA only in the cytoplasm as cytoplasmic DNA (Ikwuka, 2023a). The discovery of antibiotics in the early twentieth century was a huge milestone achievement, especially for therapeutic and prophylactic purposes against a variety of bacterial infections. However, with time, some of these microorganisms continued to become resistant to these antibiotics. This resistance poses a huge threat to antibiotic therapy and puts a substantial clinical and financial burden on the healthcare system. The major cause of this phenomenon as observed by (Arlet, 2006) is the spread of plasmid-encoded Extended-Spectrum Beta -Lactamase (ESBL) genes, conferring resistance to third generation cephalosporins introduced into the

clinical practice in the early 1980s as a major breakthrough in the fight against beta-lactamase-mediated bacterial resistance.

β-lactamases possess the ability to breakdown third-generation cephalosporins and aztreonam; and yet are inhibited by clavulanate. Moreover, ESBL-producing organisms also manifest co-resistance to many other classes of antibiotics, resulting in limited treatment option (Rawat, 2010). It has been established that chronic metabolic disorders have the ability to compromise immunity due to the activetion of different systemic, immune inflammatory processes. Metabolic disorders e.g. Hypertension, Adiposity, Diabetes mellitus and Dyslipidemia collectively known as Metabolic Syndrome Diseases (MSDs) are diseases related to one another and have very high morbidity and mortality rates (Ikwuka, 2015; Ikwuka, 2017a; Ikwuka, 2017c; Ikwuka, 2023c; Ikwuka, 2023f; Virstyuk, 2016). Results obtained from different researches have shown that hypertension, diabetes mellitus, adiposity and dyslipidemia, asymptomatic hyperuricemia, systemic immune inflammation activation and fibrogenesis, can lead to kidney damage (Ikwuka, 2017d; Ikwuka, 2017e; Ikwuka, 2018a; Ikwuka, 2018c; Ikwuka, 2018d; Ikwuka, 2019a; Ikwuka, 2019c; Ikwuka, 2022; Ikwuka, 2023d; Virstyuk, 2017a; Virstyuk, 2018a; Virstyuk, 2019; Virstyuk, 2021a; Virstyuk, 2021b). Published research on ESBLs has now originated from more than 30 different countries, reflecting the truly worldwide distribution of ESBL-producing organisms. Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis (Rawat, 2010). Linked with the induction of oxidative stress are major free radicals. Among these major free radicals, superoxide anion, hydroxyl radical, and hydroperoxyl radical are of physiological significance. Non-radical of physiological significance is hydrogen peroxide (Ikwuka, 2023b, Ekechi, 2023a; Ama, 2023).

It has been noted that the treatment of choice for serious infections due to ESBL-producing organisms are Carbapenems, yet isolates resistant to Carbapenems have been reported recently (Rawat, 2010). ESBLs show an excellent example of the ability of gram-negative bacteria to develop novel resistance mechanisms to antibiotics mechanisms especially in the start of new antibiotic (Rawat, 2010). The rapid spread of ESBL-producing bacteria has gradually become of global importance, necessitating prompt actions such as continuous monitoring systems and thus, there is need for effective control of infections and public health practices which prevent the spread of infections. Change of antibiotics in order to reduce the spread of antimicrobial resistance is also a considerable intervention strategy (Rawat, 2010). Use of antibiotics that kill bacteria in the colon which produces Vitamin K can lead to Vitamin K deficiency, which in turns results in decreased production of clotting factors II, VII, IX and X. Implicated antibiotics are broad-spectrum antibiotics e.g. fluoroquinolones, cephalosporins and other penicillin derivatives (Ikwuka, 2023e). Patients with Vitamin K deficiency are prone to different degrees of bleeding which can lead to anemia (Musa, 2023; Inya, 2023a; Inya, 2023b). Nevertheless, there is also need for new and effective treatment options in patients with Metabolic Syndrome Diseases. Sodium-Glucose Linked Transporter 2 (SGLT-2) inhibitors e.g. Dapagliflozin and Glucagon-like Peptide 1 Receptor Agonists (GLP-1 RAs) e.g. Liraglutide have been found to improve the efficacy of treatment and clinical course of type 2 diabetes mellitus and hypertension in patients with such comorbidities (Ikwuka, 2017b; Ikwuka, 2018b; Ikwuka, 2019b; Ikwuka, 2021; Virstyuk, 2017b; Virstyuk, 2018b; Virstyuk, 2018c). It has also been documented that coconut water has hepatorenal protective functions in alloxan-induced type 1 diabetes mellitus (Ekechi, 2023b). Not so much information is available for molecular detection of ESBL producing *S. typhi* isolates in Nigeria. Therefore, this research was aimed at molecular detection of extended-spectrum beta-lactamase (ESBL) producing *S. typhi* from stools of patients attending Bingham University Teaching Hospital, Jos, Plateau State, Nigeria.

MATERIALS AND METHODS:

Study Setting

The study was carried out at Bingham University Teaching Hospital (BhUTH), formerly ECWA Hospital. BhUTH is located around Zaria bypass in Jos, Plateau State, North-Central Nigeria. BhUTH is a missionary-owned hospital, established in 1959

with a 250 bed capacity. Jos is the capital city of Plateau State with current metro area population of 1,001,000 in 2024, a 3.2% increase from 2023. Jos is situated at about 1,238 meters or 4,062 feet above sea level.

Sample Collection

A total of 353 stool samples were collected from suspected typhoid fever patients attending the Bingham University Teaching Hospital, Jos, Plateau State into labeled, sterile universal containers. The samples were collected between March to May 2020. The samples were carried to the Microbiology Laboratory of the same hospital and analyzed accordingly. **Table 1** shows the primers, primer sequences with amplicon sizes for extended-spectrum beta-lactamase genes.

Table 1: Primers, primer sequences with amplicon sizes for extended-spectrum beta-lactamase genes.

S/No.	Primer	Primer Sequence	Amplicon Size (bp)
1.	*bla*TEM	5´-TCGGGGAAATGTGCGCG-3´	
		5´-TGCTTAATCAGTGAGGCACC-3´	972
2.	*bla*SHV	5´-GGGTTATTCTTATTTGTCGC-3´	
		5´-TTAGCGTTGCCAGTGCTC-3	615
3.	*bla*CTX-M	5´-ACGCTGTTGTTAGGAAGTG-3´	
		5´-TTGAGGCTGGGTGAAGT-3´	857

Isolation of *Salmonella typhi*

S. typhi were isolated from stool samples of the patients using the following procedure described by (Cheesbrough, 2011; Ahmed *et al.,* 2021). Samples from the patients were collected in sterile universal bottles. A thick suspension of the sample was placed in about 1ml of sterile Selenite F broth and incubated. A loopful of 24 hours old growth from the Selenite F broth was emulsified on freshly prepared XLD agar and SS agar plates; and were incubated aerobically for 24 hours at 37^0C.

Identification of *Salmonella typhi*

Salmonella typhi isolates were identified using cultural, morphological and biochemical methods (see **Table 2**) and confirmed by PCR.

Table 2: Cultural, morphological and biochemical authentication of *S. typhi* isolated from stool of patients.

Cultural characteristics				Biochemical characteristics					Inference
	Morphological Characteristics	Gram reaction	Mot	Ind	Cat.	Cit.	TSI	H_2S	
Colourless colonies on SSA with black centers and black metallic sheen on XLD	Rod	-	+	-	+	-	+	+	*S. typhi*

+ = Positive; **-** = Negative; K = Alkaline; A = Acid; Butt. = Button; H_2SO_4 = Hydrogen Sulphate; Mot = Motility; Ind = Indole; Cat. = Catalase; Cit. = Citrate; TSI = Triple Sugar Iron

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates were carried out as described by Clinical and Laboratory Standards Institute (CLSI, 2017). Three (3) pure colonies of the isolates were inoculated into 5ml sterile 0.85% (w/v) NaCl (normal saline). Turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5ml of 1.172% (w/v) $BaCl_2$. H_2O was added into 99.5ml of 1% (w/v) H_2SO_4. A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were placed aseptically at the center of the plates and allowed to stand for 1 hour for pre-diffusion. The plates were incubated at 37ºC for 24 hours. Results were read using Kirby Bauer interpretation chart, with zone of inhibition size ≤ 14mm indicating resistance.

Determination of Multiple Antibiotics Resistance (MAR) Index

The MAR Index was determined according to the method of (Krumperman, 1983). From the result of

the antibiotics susceptibility test, MAR Index was mathematically calculated as follows:

$$\text{MAR Index} = \frac{\text{No. of antibiotics to which isolate is resistant}}{\text{Total no. of antibiotics tested}}$$

Phenotype Confirmatory Test for Extended-Spectrum Beta-Lactamase Production

The phenotypic confirmatory test for ESBL production by isolates jointly resistant to both third generation cephalosporins (Ceftazidime and Ceftriaxone) and Amoxicillin-Clavulanic acid were carried out using Double-Disc Synergy Test (DDST) method earlier described by (Jarlier, 1988). On sterile Mueller-Hinton agar plates and Amoxicillin-Clavulanate (30µg) disc placed at the center of the plate, 105cfu/ml bacterial suspension was streaked. Ceftriaxone (30µg) and Ceftazidime (30µg) discs were then placed 15mm (edge-to-edge) from the center disc. Enhancement of inhibitory zone in the area between the disc and any one of the β-lactam discs were compared with inhibitory zone on the far side of the drug disc and interpreted as indicative of the presence of an ESBL in the tested strain. For ESBLs production, *Klebsiella pneumoniae* was used as positive control while *Escherichia coli* were used as negative control.

DNA Extraction

Boiling method was used for DNA extraction as described by (Abimiku, 2016). Bacterial DNA was isolated from a 24-hour culture in Lysogeny broth (Luria-Bertani broth/LB broth) and was prepared according to the manufacturers' protocol, after purification on MacConkey agar. Centrifugation at 3,200 rpm in a micro-centrifuge for 2 minutes at room temperature yielded the bacterial cells and the supernatant was thrown away. The bacterial cells were re-suspended in 1ml of sterile normal saline and the micro-centrifuge tubes were placed in the vortex for 5 seconds. Centrifugation was repeated at 3,200 rpm for 1 minute and the supernatant was then thrown away again. 0.5ml of sterile normal saline was added to the pellets and the tubes were vertexed for 5 seconds after which they were heated in the block heater at 90°C for 10 minutes. Following heating, rapid cooling was done by transferring the tubes into the freezer for 10 minutes. After centrifugation was done at 3,200 rpm for 1 minute, cell debris were removed. 300µl of the supernatant were transferred into a sterile 2ml appended tube as DNA and stored at -10°C until use.

DNA Amplification of Extended-Spectrum β-Lactamase Genes

In order to amplify the ESBL genes present in the isolates, Multiplex Polymerase Chain Reaction (PCR) was done. Using previously published primer sets and conditions, the presence or absence of *bla*CTX-M, *bla*SHV and *bla*TEM genes were tested for. **Table 1** contains the list of the primer sequences and expected amplicon size for each gene. The reactions were carried out in 20µl reaction volume made up of 10µl of Mastermix (Qiagen), 0.32µl of primers (0.16µl each of forward and reverse primers), 3µl of DNA and 6.68µl of nuclease free water. The primer concentration reached at 0.2M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine closed. Conditions during the reactions were set as: 3 minutes of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 40 seconds, initial extension at 72°C for 50 seconds, final extension at 72°C for 3 minutes and a hold at 4°C indefinitely. The amplified bands were visualized under ultra-violet light and photographed. Reaction mixtures without a DNA template served as negative controls (Fontana, 2003; Rahman *et al*., 2019).

Statistical Analysis

All statistical analyses were carried out using PAST and Microsoft EXCEL for Windows version 21.0.

Ethical Approval

Ethical approval for this study was received from the Health Research and Ethical Committee of Bingham University Teaching Hospital, Jos with Reference No.: NHREC/21/05/2005/00706. Voluntary verbal consent was gotten from all study participants after informed decision. Guardians served the purpose of consent for patients below 16 years of age.

RESULTS:

This study recorded the occurrence of 37(10.5%) *Salmonella typhi* isolates out of three hundred and fifty-three (353) stool samples collected from patients suspected to have typhoid fever and attending Bingham University Teaching Hospital, Jos.

Cultured phenotypic identification of the isolates showed milkfish single colonies with black center on SSA and black metallic sheen on XLD which were Gram Negative, rod shape. Biochemical tests reviewed motile, hydrogen sulphate production and indole negative, citrate negative, triple sugar iron test, with H_2SO_4 production. Some isolates which produced gas were identified as *S. typhi* (see **Table 2**). The antibiotic resistance patterns of the isolated 37(10.5%) *S. typhi* are presented in **Table 3**.

Table 3: Antibiotic resistance patterns of *S. typhi* isolated from stool of patients, *n*=37.

Antibiotic	Disc Content (µg)	No. (%) Resistance
Amoxicillin (AML)	20	35(94.5)
Amoxicillin-Clavulanate (AUG)	30	37(100)
Cefuroxime (CRX)	30	9(24.3)
Ceftazidime (CAZ)	30	25(67.5)
Ceftriaxone (CTR)	30	29(78.3)
Chloramphenicol (CH)	30	18(48.6)
Ciprofloxacin (CPR)	5	22(59.4)
Gentamicin (GEN)	10	20(54.0)
Ofloxacin (OFL)	5	20(54.0)
Ampicillin (AMP)	10	37(100)
Erythromycin (ERY)	30	23(62.1)
Nitrofurantoin (NIT)	300	0(0)
Streptomycin (STR)	30	16(63.2)

All the 37 isolates showed resistance (i.e. 100% resistance) to Augmentin and Ampicillin while Nitrofurantoin was observed to be effective on the 37 *S. typhi* isolates (i.e. 100% susceptibility). The antibiotic resistance patterns of the other antibiotics include Ceftriaxone (78.3%), Amoxicillin (94.5%), Erythromycin (62.1%), Ceftazidime (67.5%), Streptomycin (63.2%), Ofloxacin (54.0%), Chloramphenicol (48.6%), Ciprofloxacin (59.4%), Cefuroxime (24.3%), and Gentamicin (54.0%). **Table 4** depicts the phenotypic resistance profile of *S. typhi* isolates from stool samples of the patients.

Table 4: Phenotypic resistance profile of *S. typhi* isolates from stool samples of patients, *n*=37.

S/ No.	Phenotype	Isolates (%)	No. of occurrence
1.	AUG-AMP-AMX	2	5.40
2.	OFL-AUG-AMP-STR	2	5.40
3.	CRX-AUG-AMP-AMX	2	5.40
4.	CRX-AUG-AMP-AMX-S	4	10.81
5.	CRX-AUG-AMP-AMX-ERY	1	2.70
6.	CRX-AUG-AMP-AMX-STR-CH	4	10.81
7.	CRX-GEN-AUG-AMP-AMX-STR-CH	1	2.70
8.	CAZ-CRX-AUG-AMP-AMX-ERY-CH	3	8.10
9.	CRX-GEN-CPR-OFL-AUG-AMP-AMX	2	5.40
10.	CAZ-CRX-AUG-AMP-AMX-STR-ERY-CH	2	5.40
11.	CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR	1	2.70
12.	CTR-CAZ-GEN-CPR-OFL-AUG-AMP-AMX-CH	1	2.70
13.	CRX-CAZ-GEN-CPR-AUG-AMP-AMX-STR-CH	1	2.70
14.	CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-CH	1	2.70
15.	CTR-GEN-CPR-OFL-AUG-AMP-AMX-STR-CH	1	2.70
16.	CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-ERY	1	2.70
17.	CTR-CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-CH	1	2.70

18.	CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-ERY-CH	1	2.70
19.	CTR-CAZ-CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-ERY	3	8.10
20.	CTR-CAZ-CRX-GEN-CPR-OFL-AUG-AMP-AMX-STR-ERY-CH	3	8.10

Key: AMP=AMPICILLIN; AMX=AMOXILLIN; AUG=AMOXYCILLIN/CLAVULANATE; CAZ=CEFTAZIDIME; CH=CHLORAMPHENICOL; CPR=CIPROFLOXACIN; CRX=CEFUROXIME; CTR=CEFRTRIAXONE; ERY=ERYTHROMYCIN; GEN=GENTAMICIN; OFL=OFLOXACIN; STR=STREPTOMYCIN

The Multiple Antibiotics Resistance Index (MAR Index) of the 37 *S. typhi* isolated from the study was determined which showed the number of isolates and occurrence of 3(8.10%) with the highest MAR Index of 0.90 while number of isolates and occurrence of 2(5.40) with the lowest MAR Index of 0.20 (see **Table 5**).

Table 5: Multiple Antibiotics Resistance Index (MAR Index) of *S. typhi* isolates from stool samples of patients.

MAR Index	No. (%) of Isolates (*n*=37)
0.20	2(5.40)
0.30	4(10.81)
0.40	9(24.32)
0.50	5(13.51)
0.60	7(18.91)
0.70	4(10.81)
0.80	3(8.10)
0.90	3(8.10)
1.00	0(0.00)

Of the 37(10.4%) *S. typhi* isolates, 5(1.4%) isolates showed ESBLs production according to the double disc synergy test (see **Fig. 1** and **Table 6**).

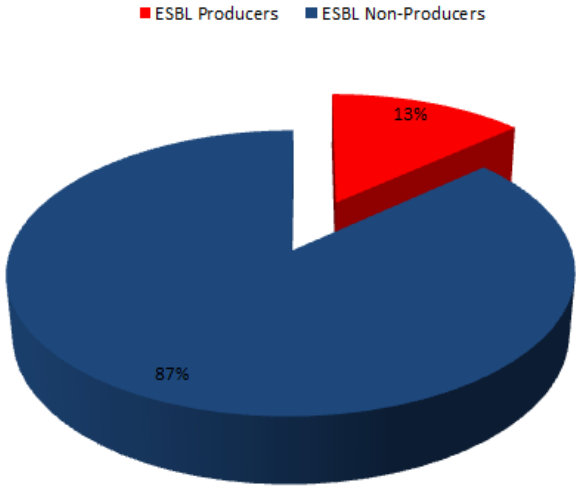

Fig. 1: Pie chart showing ESBL-producing *S. typhi* isolated from the patients.

Table 6: Phenotypic ESBL production in antibiotic resistant *Salmonella typhi* from stool samples of patients.

Isolates	No. of occurrence (%) ESBL negative	No. of occurrence (%) ESBL positive
B-lactam resistant *S. typhi*	32(86.5)	5(13.5)

Among the isolates, ESBL producers showed remarkably more resistance against all the antibiotics used in this study. The ESBLs producers include samples 2 and 16 with 92.3% and 84.6% respectively while samples 6, 8, and 18 had 69.2% each as their resistant percentages (see **Fig. 2** and **Table 7**).

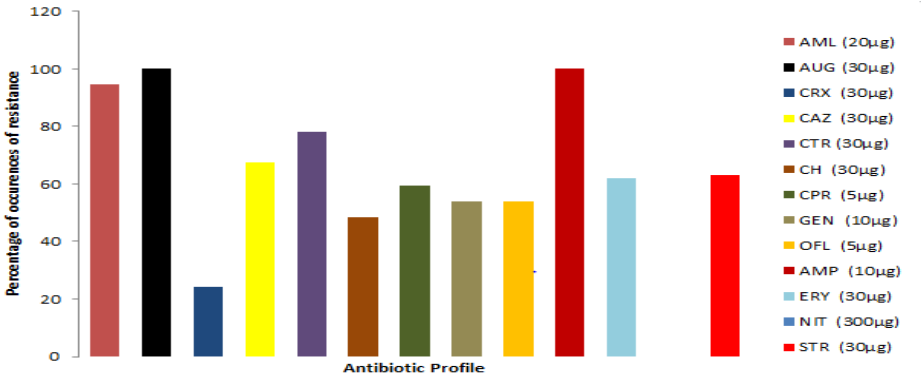

Fig. 2: Antibiotics resistance profile of *S. typhi* isolated from stool of patients.

Table 7: Phenotypic detection of ESBLs production in *S. typhi* isolates from stool of patients.

S/No.	Isolate ID	ESBLs Production
1.	BHU 1	-
2.	BHU 2	+
3.	BHU 3	-
4.	BHU 4	-
5.	BHU 5	-
6.	BHU 6	+
7.	BHU 7	-
8.	BHU 8	+
9.	BHU 9	-
10.	BHU 10	-
11.	BHU 11	-
12.	BHU 12	-
13.	BHU 13	-
14.	BHU 14	-
15.	BHU 15	-
16.	BHU 16	+
17.	BHU 17	-
18.	BHU 18	+
19.	BHU 19	-
20.	BHU 20	-
21.	BHU 21	-
22.	BHU 22	-
23.	BHU 23	-
24.	BHU 24	-
25.	BHU 25	-
26.	BHU 26	-
27.	BHU 27	-
28.	BHU 28	-
29.	BHU 29	-
30.	BHU 30	-
31.	BHU 31	-
32.	BHU 32	-
33.	BHU 33	-

34.	BHU 34	-
35.	BHU 35	-
36.	BHU 36	-
37.	BHU 37	-

Key: + = Positive (ESBLs Present); - = Negative (ESBLs Absent)

Genotypic frequency of ESBL resistance genes in the phenotypic ESBL producing isolates showed the occurrence of *bla*CTX-M 2(40.0%), *bla*SHV 3 (60.0%), and *bla*TEM 5(100.0%) and were detected on base pairs 857, 615, and 972 respectively. The occurrence of co-carriage *bla*TEM/*bla*SHV (60.0%) was high while the occurrence of *bla*SHV/*bla*CTX-M, *bla*TEM/*bla*CTX-M, and *bla*SHV/ *bla*TEM/ *bla*CTX-M were 40.0% as shown in **Table 8**.

Table 8: Genotypic occurrence of Extended-spectrum beta-lactamase genes in phenotypic ESBL producing *S. typhi* from stool samples of patients.

ESBL genes	Number of occurrence (%) of *S. typhi* (*n*=5)
*bla*CTX-M	2(40.0)
*bla*SHV	3(60.0)
*bla*TEM	5(100.0)
*bla*TEM/*bla*SHV	3(60.0)
*bla*SHV/*bla*CTX-M	2(40.0)
*bla*TEM/*bla*CTX-M	2(40.0)
*bla*SHV/ *bla*TEM/*bla*CTX-M	2(40.0)

Classes of antibiotics resistance of *S. typhi* from stool samples of patients (see **Table 9**) was determined which showed occurrence (%) of 27(72.97) for Multidrug Resistant (MDR), 10(27.02) were extensively drug resistant (XDR) while none of the isolates (i.e. 0(0.00)) had non-multidrug resistant or pan drug resistant.

Table 9: Classes of antibiotics resistance of *S. typhi* isolated from stool samples of patients.

Phenotype	Occurrence (%) (*n*=37)
MDR	27(72.97)
NMDR	0(0.00)
PDR	0(0.00)
XDR	10(27.02)

Key: MDR=Multidrug Resistant; NMDR=Non-Multidrug Resistant; PDR=Pan Drug Resistant; XDR=Extensively Drug Resistant

The DNA bands of amplified ESBLs genes in phenotypic ESBL production is shown in **Fig. 3** whereas the distribution patterns of ESBL genes on agarose gel electrophoresis is shown in **Table 10**.

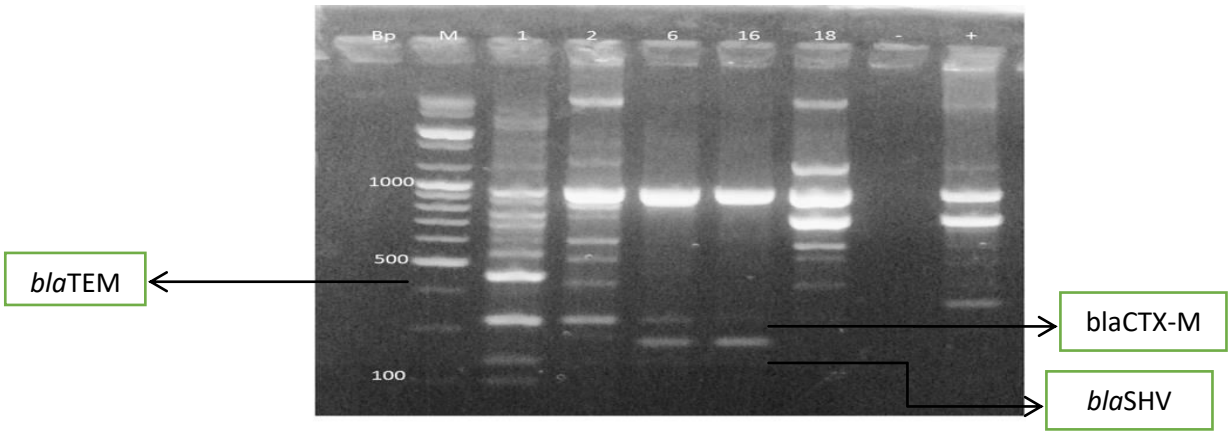

Fig. 3: Agarose gel electrophoresis of the amplified *bla*TEM, *bla*CTX-M, and *bla*SHV genes from the ESBL producing *S. typhi* isolates.

Table 10: Distribution patterns of ESBL genes on agarose gel electrophoresis.

Sample	*Bla*TEM	*Bla*SHV	*Bla*CTX-M
2	+	-	-
6	+	+	+
8	+	-	-
16	+	-	-
18	+	+	+

Key: **+** Positive; - Negative

DISCUSSION:

Third generations cephalosporins remain the most commonly prescribed class of antibiotics for case management of typhoidal and non-typhoidal salmonellosis in many countries of the world, including Nigeria (Akinyemi, 2014; Arlet, 2006). However in this study, the focus was placed on only typhoidal salmonellosis caused particularly by *S. typhi*. An increasing incidence of antibiotic resistance among *Salmonella* strains worldwide has been attributed to the unapproved and indiscriminate use of antibiotics particularly in the developing countries (Mathew, 2007). Of recent, ESBLs have become a notable driver of drug resistance. Despite extensive distribution of ESBLs, the prevalence and phenotypic characteristic among clinical isolates may differ in different geographical zones (Bradford, 2001). In addition, the prevalence of ESBLs between *E. coli* and *Klebsiella* has been frequently shown in many countries, but the emergence of ESBLs in *Salmonella*, which now confers serious clinical problem is noteworthy. These isolates were subjected to ESBLs testing because they were multi-drug resistant which only 5(1.4%) isolates showed positive while 32(9.0%) isolates were negative. Not many studies have been done on extended-spectrum beta-lactamase (ESBLs) producing *S. typhi* in Jos, Plateau State. Hence, there is limited information and data on previous studies. Finding from this present research shows the importance of *S. typhi* as a potential public health burden in Jos and the necessity of continuous surveillance. This study revealed a prevalence of 10.4% *S. typhi* isolates from stool samples of patients presenting with typhoid-like symptoms in BhUTH, Jos, Plateau State, Nigeria, which is low compared with the results of other studies e.g. 24.56% noted in West Bengal, India (Das, 2012), 32.1% observed in North Karnataka, India (Metri, 2011), and 53% seen in Mumbai (Rudresh, 2011). Nonetheless, a recent research in Bangladesh noted that the distribution of *S. typhi* shows remarkable seasonality, with higher prevalence seen during the raining season (May to October) (Ahmed, 2014). Thus, the low prevalence of *S. typhi* noted in this present study could be explained by the fact that the period of samples were collected during the dry season (March 2020 to May 2020). Phenotypic confirmatory test method also showed a 1.4% prevalence rate of ESBLs producing *S. typhi* in this study. A limited number of studies have been carried out to ascertain the prevalence of ESBLs producing *Salmonella* strains with zero to very low prevalence rates. In addition, resistance to antibiotics was remarkably higher among ESBL producers than some non-ESBL producers (see **Table 3**). Ceftazidime and Ceftriaxone resistance in this study were found to be significantly linked to ESBLs production in the isolates. This agrees with the recent finding which showed that most detected ESBLs have special affinity to degrade Ceftazidime (Bradford, 2001). Another study has also reported Ceftazidime to be efficient in screening isolates as potential ESBL producers (Vahaboglu, 2001).

Of interest in this study is the fact that some of the bacteremic strains of *S. typhi* that produced these ESBL genes can be used to explain why continuous fever occurs in affected patients despite them receiving treatment. The resultant consequence of this is the potential for spread of emerging *bla*TEM, *bla*SHV, and *bla*CTX-M producing *S. typhi* in Jos and its environs which will increase the prevailing public health burden. Continuous surveillance of *bla*TEM-producing pathogens is requires to guide prophylactic strategies in Nigeria generally. *bla*TEM gene cluster carriage has been linked with increased resistance to Ceftriaxone. Thus, the clinical practice of switching *Salmonella* bacteremic and febrile patients who failed treatment with Ceftriaxone and Ceftazidime empirically to Cefuroxime also possess the risk of treatment failure in this environment. For these patients, this present study recommends the

use of either Levofloxacin, Imipenem, Azithromycin or Nitrofurantoin for their treatment. In treating multidrug-resistant infections, the clinical effectiveness of these antibiotics has been reported in Tanzania (Mshana, 2011), in Nepal (Pokharel, 2006), and in India (Pathak, 2012). These antibiotics are also included in the drug formulary of many hospitals in Lagos State and other states in Nigeria, where they are used for treatment of in-patients and out-patients affected by other bacterial infections (Aibinu, 2003; Akinyemi, 2014). In addition to the results of the conjugation experiment revealing that the ESBL genes carried by *S. typhi* was plasmid-mediated, the results also showed the potential for fast spread of this genetic marker to other members of *Enterobacteriaceae* such as *E. coli*, which are often seen in polymicrobial infections in Plateau State and other states of Nigeria. Similar plasmid-mediated transfer of *bla*TEM, *bla*SHV, and *bla*CTX-M resistance to antibiotics such as Cefuroxime, Aminoglycosides, and beta-lactamase inhibitor-containing antibiotics such as Augmentin, as demonstrated in this present study, have been documented by previous researchers from other countries of the world (Mshana, 2011; Jin, 2006; Bado, 2012; Fischer, 2014). Moreover, additional antibiotic resistance conferred by this genetic marker may also lead to the emergence of pan-resistant *S. typhi* in Nigeria, as was recently reported in some Asian countries (Tadesse, 2014). The spread of ESBL-producing *S. typhi* to other states in Nigeria and other neighboring countries is also a possibility.

CONCLUSION:

The incidence of infections with *S. typhi* is growing worldwide especially in poor developing countries where clean water supply is scarce and unhygienic sanitary conditions prevail. The prevalence of *S. typhi* from stool samples of patients in this study is relatively low. However, subsequent detection of extended-spectrum beta-lactamase (ESBL) genes in the isolates has proven to be a potential serious public health issue and almost all the antibiotics tested were not effective against the isolates except Cefuroxime and Nitofurantoin. Hence, these two drugs should be considered for typhoid infection treatment. Continuous, vigorous and collaborative measures such as public health education and mass surveillance will help to further deepen the knowledge on the mode of transmission, prevention, treatment and evolution of these resistant isolates.

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CONFLICTS OF INTEREST:

All the authors of this manuscript agreed that they have no confliction to make the manuscript publishable.

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